UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/522,826	07/21/2005	Viktor Menart	LB/G-32991A/LEK	4902

83721 7590 08/25/2009

Lek (Slovenia) - LUEDEKA, NEELY & GRAHAM, P.C.
P.O. BOX 1871
Knoxville, TN 37901

EXAMINER	
STOICA, ELLY GERALD	

ART UNIT	PAPER NUMBER
1647	

MAIL DATE	DELIVERY MODE
08/25/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/522,826	MENART ET AL.	
	Examiner	Art Unit	
	ELLY-GERALD STOICA	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *05 May 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4-7,10,12-17,19-21,23-26 and 38-42* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4-7,10,12-17,19-21,23-26 and 38-42* is/are rejected.

7)☒ Claim(s) *1* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendment

Status of the claims

1. In the amendment filed 05/05/2009 Applicant cancelled claim 3, amended claims 1, 21, 26 and 38, and added the new claims 40-42. Claims 1, 4-7, 10, 12-17, 19-21, 23-26, and 38-42 are pending in the application and currently examined.

Withdrawn claim rejections

2. The scope of enablement rejection of claims 1, 3, 5-7, 10, 12-17, 19-21, 23-26, 38-39 under 35 U.S.C. 112, first paragraph, is withdrawn in view of persuasive arguments presented on page 4 of the Remarks.

3. The rejection of claims 1, 5, 6, 10, 12-17 and 23-25 under 35 U.S.C. 102(e) as being anticipated by Gonzales-Villasenor, LI (U.S. Pub. No.20030166062, filed 02/22/2002) is withdrawn in view of amendments to the claims.

4. The rejection of claims 1, 5, 6, and 12-14 under 35 U.S.C. 102(b) as being anticipated by Li et al. (U.S. Pat. 5,912, 327) is withdrawn in view of amendments to the claims.

5. The rejection of claims 1, 5-7, 12-14, 16, 17 and 23-26 under 35 U.S.C. 102(b) as being anticipated by Patra et al. (Protein Expression and Purification 18,182–192, 2000) is withdrawn in view of amendments to the claims.

6. The rejection of claims 1, 5, 6, 12-14, 16, 17, 23 and 25 under 35 U.S.C. 102(b) as being anticipated by Panda et al. (J. Biotechnology, 75, 161-172, 1999) is withdrawn is withdrawn in view of amendments to the claims.

Maintained and new claim objections and rejections

Claim objections

7. Claims 1 is objected to because of the following informalities: the claim contains non-elected subject matter. In the response to the election/ Restriction requirement filed 08/24/2007, Applicant elected without traverse the specie G-CSF. The amended claim 1 now contains multiple non-elected species together with the elected specie. Appropriate correction is required.

Claim Rejections - 35 USC § 112

8. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 4-7, 10, 12-17, 19-21, 23-26, 38-39 remain and the new claims 40-42 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, in the independent claim 1, it is unclear how the "principle of performing the fermentation" can be regulated so as to perform the method step claimed. As such, the metes and bounds of the claim cannot be determined.

On page 3 of the Remarks Applicant argues that: "Regarding the "principle of performing the fermentation", pages 13-14 of the specification teach the meaning of this phrase and how the same may be regulated." The arguments were carefully considered but not found persuasive because it is considered that one cannot regulate the principles of a process but can choose different types of performing that process. If Applicant intended to claim different types of performing the fermentation it would be helpful to claim it as such.

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

10. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

11. Claims 1, 4-7, 10, 12-17, 19-20, 23-26, and 38-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Patra et al. (Protein Expression and Purification 18,182–192, 2000) in view of Panda et al. (J. Biotechnology, 75, 161-172,1999) and in

further view of Souza LM (U.S. Pat. No. 4,810,643), Ambrosius et al. (U.S. Pat. No. 5,618,927), Camble et al. (U. S. Pat. 5,773,581), and Pelleymounter et al. (U.S. Pub. 20020009798).

The independent claim1 is drawn to a process for the production of a biologically active protein (such as G-CSF), comprising:

expressing said protein as a heterologous protein in an expression system comprising a cultivated organism having one or more cells, wherein the protein is expressed as a substantially correctly folded protein precursor in non-classical inclusion bodies;

regulating one or more cultivation parameters selected from the group consisting of temperature of cultivation, composition of cultivation medium, induction mode, principle of performing the fermentation, addition of a stress induction agent, and co-expression of auxiliary proteins, wherein regulating the one or more parameters increases the proportion of substantially correctly folded protein precursor present in the inclusion bodies in the cells, relative to the proportion of substantially correctly folded protein precursor present in inclusion bodies in cells of an organism not cultivated by regulating said parameters;

isolating the inclusion bodies from the cells of the .organism; optionally, washing the inclusion bodies;

solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the non-classical inclusion bodies with a non-denaturing aqueous solvent having a pH of about 8.0;

and purifying the biologically active protein from the solubilized substantially correctly folded protein precursor wherein the process for the production of the active protein is free from any denaturation and renaturation of the protein.

The process of production may be performed in yeast or bacteria and the proportion of the heterologous protein to the total protein mass of the cell is at least higher than 10% and as high as 30%. The temperature of cultivation ranges from 20°C to 30°C.The inducer may be IPTG or lactose or NaCl and present in a concentration from 0.1mM to 1 mM. The inducer is added at the beginning of the fermentation, which may be in a batch mode, fed batch mode in one or more flasks. The limitations that the claims 19-20, 23-26, 38 and 39 add consist of specific fermentation media (GYST, GYSP, LYSP LYST, LBON or GYSPON) and the use of non-denaturating solution of N-Lauroyl sarcosine in ranges of about 0.1% to about 0.25% mass per volume and a high solubilizing concentration of a buffer which may be HEPES. Further limitation is claimed as a minimal specific activity of G-CSF of 10^7 IU/mg.

Patra et al teach methods of obtaining recombinant human growth hormone (r-hGH) from E. Coli inclusion bodies. The Inclusion bodies from the cells were isolated and purified to homogeneity. Various buffers with and without reducing agents were used to solubilize rhGH from the inclusion bodies. Complete solubilization of the inclusion bodies was obtained with Tris/HCl buffer at pH about 8 (8.5) containing 2M urea. Biologically active GH was obtained after further purification by ion-exchange chromatography. The E.Coli in which the heterologous protein was expressed was grown in a 3.5 l fermentor in a fed-batch mode with a continuous supply of glucose and

yeast extract (at 10g/L) and the culture was induced with 1 mM IPTG. The inclusion bodies were washed with Tris-HCl buffer and with water. The solubilizing of the hGH from the inclusion bodies was performed with 2M Tris buffers at pH about 12 with or without 2M Urea (Material and Methods). The level of expression of r-hGH was around 13% of the total cellular protein (Results, first full paragraph).

Patra et al. are silent about purifying G-CSF, the temperature of cultivation, the composition of the cultivation medium as being GYST or GYSP or using N-lauroyl sarcosine.

Panda et al. teach a process for maximizing the volumetric productivity of recombinant ovine growth hormone (r-oGH) expressed in Escherichia coli during high cell density fermentation process. Kinetics of r-oGH expression as inclusion bodies and its effect on specific growth rates of E. coli cells were monitored during batch fermentation process. It was observed that during r-oGH expression in E. coli, the specific growth rate of the culture became an intrinsic property of the cells which is reduced in a programmed manner upon induction. Nutrient feeding during protein expression phase of the fed-batch process was designed according to the reduction in specific growth rate of the culture. By feeding yeast extract along with glucose during fed-batch operation, high cell growth with very little accumulation of acetic acid was observed. Use of yeast extract helped in maintaining high specific cellular protein yield which resulted in high volumetric productivity of r-oGH (abstract). The cultures were added IPTG at 1mM concentration in various stages of the fermentation process (Table 2). The inclusion bodies formed were separated from the E. Coli cells and washed with

water and contained monomeric recombinant ovine Growth Hormone in proportion of 95%. They were solubilized in Tris-HCl buffer containing a non-denaturating concentration (1%) of Sodium Dodecyl Sulfate (Material and Methods). Panda et al. present, in table 1, the composition of their medium. The composition is not the same as the GYST, GYSP, LYSP LYST, LBON or GYSPON. In the specification of the instant Application the composition of the media is described ([0074]). One of the buffers (GYST) differs from the classic E. Coli growth medium LB by containing 10g/l glucose and metals in traces. The medium taught by Panda et al. contains 10g/l glucose and metal in traces. This shows that it was routine in the art to adapt the composition for the bacterial growth media to accommodate various growth conditions.

Souza et al. teach E. Coli expression of human recombinant G-CSF by cultivation of the cells in LB broth and by modulating the temperature (col. 16, lines 1-15). The density of growth is low though and the cells contained between 3-5% G-CSF.

Ambrosius et al. teach solubilization and renaturation steps are necessary in order to convert proteins are produced in prokaryotic cells such as E. coli, (in inclusion bodies) into their active form. The process according to the present invention can be carried out in one of two ways. One variant is to work with a Tris buffer of the stated concentration so that Tris or/and a Tris salt is also used for adjusting the pH. The second variant is to work with a buffer which has previously been described for the corresponding process and to additionally add Tris or/and a salt of Tris. This means that the pH value of the incubation solution is adjusted by a buffer substance which is different from Tris. In both cases it is expedient to take care that the addition of Tris or

the increase in Tris concentration does not result in a change in pH. The process according to the present invention comprises the incubation of the inclusion bodies with a Tris solution which has a concentration of between 400mM to 2M. Examples of proteins to be treated include G-CSF. The advantages of the reactivation process according is an increase in the final yield of active protein of 30 to 300% compared to a process in which a buffer is used at a lower concentration (col. 2, line 47 to col.3, line 36).

Camble et al. teaches producing G-CSF from an inclusion body by suspending said inclusion body in a detergent, particularly N-lauroyl sarcosine in salt form (e.g. Sarkosyl) at concentrations well bellow 1%, and as low as 0.2%, which will make the final removal of the detergent for obtaining an active protein easier (col.10, lines 12-55).

Pelleymounter et al. teach the use of HEPES buffer together with N-lauroyl sarcosine for processing inclusion bodies containing murine OB protein.

It is considered that the limitation added by the claim 40, namely the specific activity of the G-CSF is at least 10^7 IU/mg, is easily achievable by purification steps that are routine in the art.

It would have been obvious for a person of ordinary skill in the art at the time that the invention was made to combine the teachings of Patra et al., Panda et al. and Souza et al. to obtain a high yield of G-CSF with a reasonable expectation of success, since Patra et al. and Panda et al. improved the growth condition for obtaining proteins in inclusion bodies and Souza et al. describe a lower yield for G-CSF using just the typical LB broth. Further, in processing the inclusion bodies it would have been obvious

to process the inclusion bodies according to the combined teachings of Ambrosius et al., Camble et al. and Pelleymounter et al. with a reasonable expectation of success since each of them added improvements over the classical processes of denaturation-renaturation proteins from inclusion bodies. A person of ordinary skill in the art is always motivated to pursue the known options within her or his technical grasp. If this leads to the anticipated success, it is likely the product not of innovation but of ordinary skill and common sense.

On page 7 of the Remarks Applicant argues that none of the references discloses the claimed limitation (from independent Claim 1) of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0" the combination cannot be said to render Claim 1 obvious.. The arguments were carefully considered but not found persuasive because while it is true that none of the references, individually, describes the situation above, when combined, the references would provide the solution for obtaining substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0.

12. Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Patra et al. (Protein Expression and Purification 18,182–192, 2000) in view of Panda et al. (J. Biotechnology, 75, 161-172,1999) and in further view of Souza LM (U.S. Pat. No. 4,810,643), Ambrosius et al. (U.S. Pat. No. 5,618,927), Camble et al. (U. S. Pat.

5,773,581), Pelleymounter et al. (U.S. Pub. 20020009798) and Donnelly et al. (U.S. Pat. No. 6,677,139).

The teachings of all the references, except Donnelly et al., were presented supra. They are silent about the use of ethanol or propanol in the growth media for E. Coli.

Donnelly et al. teach methods for the production of proteins bacterial cells, methods which use a fusion protein comprising a chaperonin binding domain in host cells induced or regulated to have increased levels of chaperonin which binds the chaperonin binding domain (Abstract). Specifically, the effect of the GroES-loop leader sequence on expression of BAX protein was evaluated in cultures that enhance the expression of E. coli chaperones in the cell. The rationale was based on the assumption that folding of expressed fusion proteins would be mediated through interaction with the chaperonin GroEL. When grown in the presence of moderate concentrations of ethanol, E. coli is known to induce higher levels of chaperones and other stress proteins (example3).

It would have been obvious for a person of ordinary skill in the art at the time that the invention was made to have modified the teachings of Patra et al., Panda et al., Souza LM, Ambrosius et al., Camble et al., Pelleymounter et al. with the teachings of Donnelly et al. with a reasonable expectation of success since the method of Donnelly was used for obtaining even a toxic protein (for E. Coli) which is correctly folded due to the chaperone protein effect. A person of ordinary skill in the art is always motivated to pursue the known options within her or his technical grasp. If this leads to the

anticipated success, it is likely the product not of innovation but of ordinary skill and common sense.

On page 8 of the Remarks Applicant argues that none of the references discloses the claimed limitation (from independent Claim 1) of "solubilizing the substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0" the combination cannot be said to render Claim 1 obvious.. The arguments were carefully considered but not found persuasive because while it is true that none of the references, individually, describes the situation above, when combined, the references would provide the solution for obtaining substantially correctly folded protein precursor from the inclusion bodies under non-denaturing conditions by contacting the inclusion bodies with a non-denaturing solvent having a pH of about 8.0.

Conclusion

13. No claims are allowed.

14. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not

mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ELLY-GERALD STOICA whose telephone number is (571)272-9941. The examiner can normally be reached on 9:00-18:30 M-Th and 9:00-18:30 alternate F.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath N. Rao can be reached on (571) 272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Christine J Saoud/

Primary Examiner, Art Unit 1647